The use of transcriptomics for biomarker development to trace anabolic hormone functions

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Outline

• Anabolic Steroid Hormones and their use in animal husbandry
• Gene Expression Biomarkers
• Study design
• Results
• Conclusions
• Perspectives
Anabolic Steroid Hormones in animal husbandry

- Important for establishment of muscle tissue
- Enhance body protein accretion
- Mobilization of fat stores
- Enhanced feed efficiency and reduced costs
- Use and misuse as growth promoters in animal husbandry
**Anabolic Steroid Hormones in animal husbandry**

- Use of specific anabolic agents is licenced in Canada, USA, Australia, South Africa...
- Since 1988: use of anabolic agents is prohibited in the EU (Directive 88/146/EEC)
- Misuse of anabolics ⇒ permanent control necessary
- Detection of hormone residues using chromatography in combination with mass spectrometry
- **Problem:** Development of new xenobiotics and administration of hormone cocktails ⇒ New sensitive test systems required
Gene Expression Biomarker

- Detection of physiological changes caused by treatment with anabolic agents
  ⇒ Development of a biomarker pattern based on changes in gene expression
- Activated steroid hormone receptors act as transcription factors
  ⇒ direct influence on gene expression
Study design

- 18 Nguni heifers (9 treated, 9 untreated)
- Treatment with Revalor H by implantation for 42 days
  - 140 mg Trenbolone acetate, 14 mg estradiol
  - Implant is placed under the skin on the posterior aspect of the ear
- Tissues for biomarker screening: liver, blood, vaginal smear (containing vaginal epithelial cells)
- Sampling of blood and vaginal smear at day 0, 2, 16 and 39 of treatment
- Liver samples obtained at slaughter (day 42)
Procedure

- Selection of target genes by screening the actual literature for steroidal effects on analyzed tissues
- Quantification of gene expression via qRT-PCR
- Normalization with reference genes
- Determination of significant regulations between treatment and control using the t-test ($p<0.05$)
- Principle Components Analysis (PCA) and Hierarchical Cluster Analysis using Genex Pro. Ver. 4.3.8 (MultiD Analyses AB, Gothenburg, Sweden)
Liver samples: 5 regulated genes

Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden
Liver samples: 5 regulated genes

Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden
Blood: 11 regulated genes
Blood: 11 regulated genes

Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden
Vaginal smear: 27 measured genes

Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden
control 9
control 8
control 2
control 7
control 3
control 4
control 5
control 1
treatment 9
treatment 3
treatment 1
treatment 8
treatment 7
treatment 5
treatment 2
circle
control 6

treatment 6

treatment 4

Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden
Vaginal smear: 27 measured genes

Genex Ver. 4.3.8, MultiD Analyses AB, Gothenburg, Sweden
Conclusions

- Potential of transcriptomics for the development of a biomarker pattern to screen for the abuse of anabolic agents
- The more regulated genes the better results using PCA or Hierarchical Clustering
- Tissues that are directly influenced by steroid hormones are better for the development of gene expression biomarkers
Perspectives

• More trials with more animals and other anabolic agents
• Large number of untreated control samples
• Combination of transcriptomics with other „omic“ technologies, like proteomics or metabolomics
Thank you for your attention!